
Stephan Bielfeldt

STEPHAN BIELFELDT*, CHRISTIANE RÖCK,
MARIANNE BRANDT, KLAUS-PETER WILHELM
*Corresponding author
proDERM GmbH, Schenefeld-Hamburg, Germany

Good measurement practice matters
Clinical sun protection product testing update

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Abstract Whilst light emitted to the earth is essential for life, overexposure to it can cause many adverse effects leading to premature actinic skin aging, photo-dermatoses, and even skin cancer. Due to the increase in the incidence of skin cancer and the public concerns of ozone layer depletion, more attention is being placed on protecting the skin from the sun's ultraviolet rays with broad spectrum sunscreens, as well as developing other prevention strategies. As described herein, since sunscreen products provide an important protective function they should always be tested with due care, using accurate and validated methodology. If an imprecise method is used in sunscreen testing, this can lead to a misunderstanding of the protective function and result in considerable damage to both consumers and producers. It is therefore understandable that a labeled SPF that is based on a wrong measurement might have significant impact on the sales figures, brand image and might even lead to indemnity claims. Therefore, the quality and the accuracy of the measurement methods in particular, are critical. In addition due care must also be given to those methods used for evaluating the longevity of sunscreen protection when exposed to instances of water, sand and sporting activities.

INTRODUCTION

Contrary to scientific fact, many people continue to believe that constant exposure to sunlight is beneficial to their general well-being and despite warnings about the dangers, most enjoy relaxing in the sun. Whilst sunlight does have many advantages, exposure to the skin may result in premature actinic skin aging, pigmentary changes, photosensitivity reactions and increased incidence of skin carcinogenesis (1). Therefore, the need for reliable sunscreen protection, to reduce the damaging effects of ultraviolet (UV) radiation to the skin cannot be dismissed (2).

Sunburn is inflammation of the skin caused by actinic rays from the sun or artificial sources (1-3), and severe exposure may result in erythema, blisters and pain. As ultraviolet rays penetrate the dermis, they break down collagen and elastin, the two main structural components of the skin, a process that results in the wrinkled appearance of sun-damaged skin (4). In addition, the sun damages the DNA of the exposed skin cells. In response, the cells release enzymes that excise the damaged parts of the DNA and encourage the production of replacement DNA (5).
At the same time, the production of melanin is stimulated and a suntan results from this attempt by the skin to protect itself (6-8). Light-skinned persons and infants are especially susceptible to ultraviolet rays because they lack sufficient protective skin pigment (9). Certain diseases and drugs may also increase photosensitivity (10).
Due to the increase in the incidence of skin cancer and the effects of ozone layer depletion, more attention is being placed on protecting the skin from the sun's ultraviolet rays with broad spectrum sunscreens or clothing (11).
Broad spectrum sunscreens block both UVA and UVB rays (the two relevant bands of ultraviolet radiation), while UVC is filtered out by the stratosphere. The relative UVB protection of a sunscreen is indicated by its SPF (sun protection factor) number; a higher number indicates a more effective sunscreen (12).
However to achieve an SPF15 or higher, protection against UVA is also required (12).

Further to the need for sun burn protection testing, there is also a concomitant need for substantiation of claims targeting UVA-protection water and sweat resistance of sunscreen products, eye safety, and resistance to sand adherence. In this paper we update the status of protective sun-care testing and describe our methods for additional claims support in terms of product benefits.

SUN PROTECTION TESTING ON THE SKIN

Since sunscreen products provide a protective function they should always be tested with due care, using the most accurate methods.
If an imprecise method is used in sunscreen testing, this can lead to a misunderstanding of the protective function and result in considerable damage to both consumers and producers. Image and sales suffer for the long-term if a product is evaluated as "unsatisfactory" or even "deficient" following market introduction. Therefore, the quality and the accuracy of the measurement methods in particular, are critical.

Ultraviolet-B Radiation (UV-B) - In the development of sunscreens there are different regulatory requirements for the testing of the protective capacity against UV. Sun-protection-factor (SPF) is assigned to protection against UVB which causes sunburn. SPF is defined as the ratio of the minimal erythema dose (MED) in sunscreen protected skin to the MED in non-sunscreen protected skin. Depending on where the product is to be sold the test methods for the determination of SPF differ e.g. FDA, and the ISO24444. The skin type of the individual is determined by either their MED, classification according to the Fitzpatrick index or skin color typing (13).

The test of sun protection factors is carried out according to the International Norm as described in the guideline of ISO 24444, November 2010 (14). In our institute, testing is carried out with a xenon arc solar simulator (300 W Multiport) equipped with guideline conforming filters. The output spectrum of our solar simulators is regularly monitored for conformity with the guideline. The number of volunteers is at least 10 in accordance with the guideline.

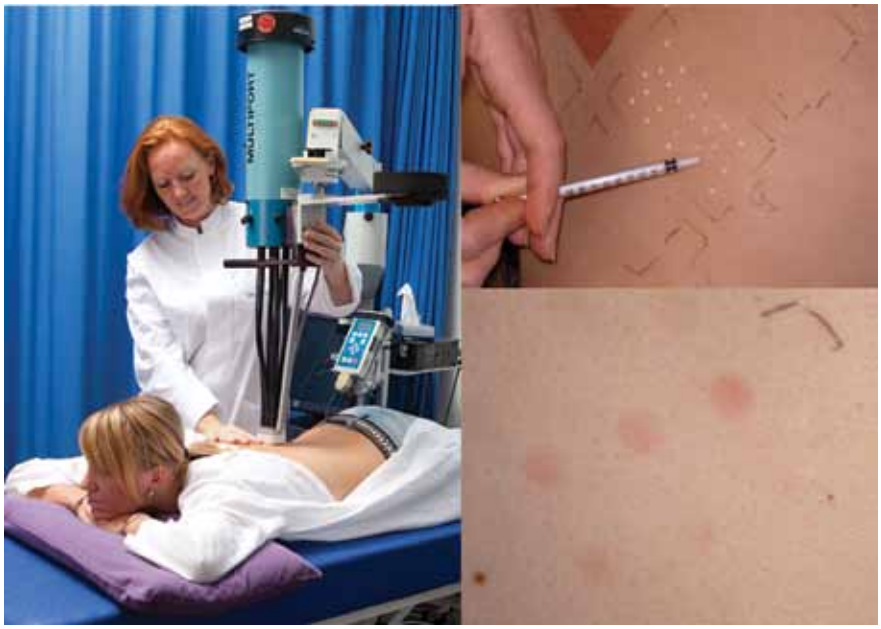

Figure 1. For SPF testing according to ISO 24444, 2 mg/cm² of product is applied to the test areas in small evenly distributed dots (upper right image). Irradiation of 6 spots per test area with increasing UV-doses is performed in prone position with a xenon arc sun simulator (left image). The minimal erythema dose (MED) on protected and unprotected test areas is read 16 to 24 hours post — irradiation (lower right image).

A sufficient number is added until the 95% confidence interval (CI) of the SPF is within ±17% of the mean value (Figure 1).
In a 2013 paper (15), Kockott et al. raise the issue of compliance with RCEE (relative cumulative erythemal effectiveness) values in defined wavelength ranges when determining in vivo SPF. This means that when testing SPF, the lamp filters need to demonstrate compliance with the strict criteria laid down by the ISO Guideline. This is not always obtained with commercially available filters, and adjustment of filters by individual adaptation of filter thickness or the use of new filter types, might be required to reach this compliance.
Ultraviolet-A Radiation (UV-A) - Good sunscreen products today also contain UVA protection, since it is known to contribute to sunburn erythema, induce photosensitivity reactions, cause pigment disorders, and enhance some photo-aging changes in the skin.
In vivo UV-A protection factors are assessed by using the in vivo Persistent Pigment Darkening (PPD) method in accordance with the Standard of Japan Cosmetic Industry Association (JCIA) of 1995 or in the EU using ISO24442. Xenon sun simulators (300 W Multiport) with appropriate filtration of the UVA spectra are used. The lamp spectrum is checked regularly by an accredited independent institute. The number of recruited volunteers is in accordance with the appropriate guideline to reach the statistical criterion. The minimal number of subjects is 10, with a maximum of 20. Screening tests are performed on 5 volunteers.

In June 2012, the European Committee for Standardization (CEN) published the standard EN ISO 24443:2012 Cosmetics – Sun protection test methods – In vitro determination of sunscreen UVA photo-protection. This standard test method replaces the earlier reference method (Guidelines - Method for in vitro Determination of UVA protection, 2011). The EN ISO test method is now considered as the reference method within the EU. Cosmetics Europe therefore recommends cosmetic manufacturers to use this standard to determine the UVA Protection Factor and the Critical Wavelength. However, the in vivo standard EN ISO 24442:2011 is still appropriate in those cases where UVA-water resistance or sweat resistance are to be determined or the in vitro method leads to inconsistent results.

WATER RESISTANCE

While sun protection factor (SPF) and UVA protection are the most important determinants of a cosmetic sunscreen product, water resistance is the third important feature.
The testing of sun protection factors and water resistance of sun protection products are carried out according to the final proposal of COLIPA (Cosmetics Europe) from their 2005 Guideline using a whirlpool procedure (16).

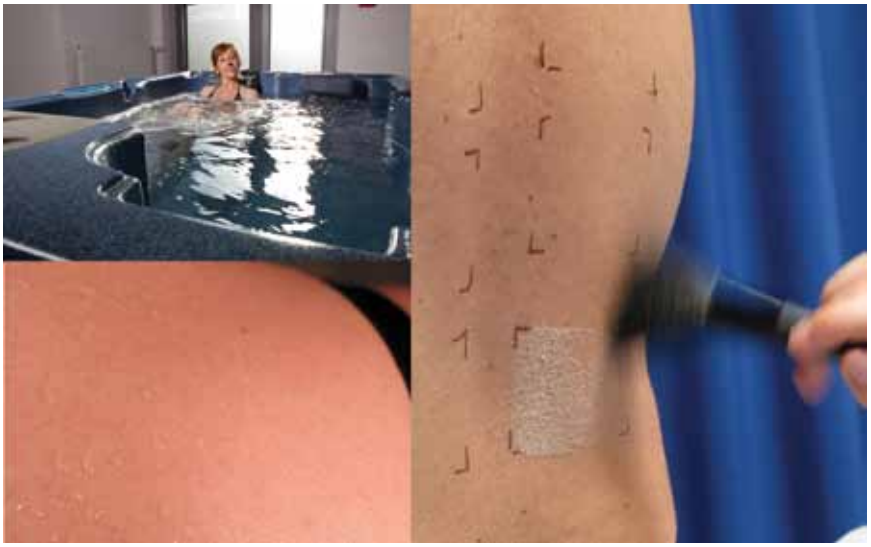

Figure 2. Water resistance according to COLIPA 2005 is assessed after defined immersion in a whirlpool (upper left image). For sweat resistance testing the volunteers stay in a sauna for approximately 15 minutes until visible sweat droplets appear on the back (lower left image). To assess how the SPF is affected by sand that might stick on the skin after applying a sunscreen on the beach, product treated test areas are exposed to sand and sticking sand is brushed away before the SPF determination (right image).

Water exposure is performed in a whirlpool water circulation system containing freshwater of 29 ± 2°C. Panelists sit in the water with their whole back under water for periods of 20 minutes with a 15 minute break after each period. A sun protection product can be claimed „water resistant" if a lower confidence limit of mean water resistance of at least 50% can be measured after 2 water periods of 20 minutes. The claim "very water resistant" is possible if a lower confidence limit of mean water resistance of at least 50% can be measured after 4 periods of 20 minutes in the water (Figure 2).

Whilst this method is the internationally accepted standard method to assess water resistance it is time-consuming and expensive. A screening method to quickly predict water resistance properties on at a lower cost is obviously a specific request of some product developers.
Several in vitro screening methods have been published but the predictive power of all these methods is limited.

We have developed an adaptation of the in vitro UVA protection method of COLIPA for a water resistance screening (17). We only recommend in vitro screening methods to pre-select from candidates which cannot all be tested in vivo. The pre-selected products can be screened in the COLIPA in vivo water resistance method with a reduced number of volunteers (usually 5) to predict water resistance. In case, the water resistance estimated in such an in vivo screening is found at about 65% or higher the study can be deemed successful and completed with further subjects to fulfil Cosmetic Europe requirements.

SWEATING RESISTANCE

Sweating is known to clearly reduce the protecting effect of sun screen products. Thus it is important to apply sweat resistant products especially during physical activities or in the hot season. We have an in-vivo method to investigate sweat resistance based on the ISO method for sun protection factor determination (18). Sweat induction is triggered by hot temperature in a sauna.

Similar to the water resistance test method of COLIPA (16) the sun protection factor is determined initially on dry skin. On another day the products are applied on the back of the subjects (different test areas) before sweating is induced in a sauna session of approximately 15 minutes at 80° C.
The time to provoke a significant sweating clearly differs from person to person. Therefore the sweat induction phase is stopped after clearly visible drops of sweat have appeared on the back. The subjects cool down at ambient temperature of approximately 24°C for at least 15 minutes before the sun protection factor is determined on the sweat exposed test areas (Figure 2).

Calculation of sweat resistance is accomplished according to the water resistance test method of COLIPA (16). In case the lower confidence limit is calculated higher than 50% of sweat resistance, a "sweat resistance claim" is justified. In studies with more than 20 test products developed for sweat resistance results showed 50% until 90% remaining preservation of sun protection after sweating.
This is comparable with the results we achieved with the water resistance test method of the COLIPA when investigating prospective water resistant products. The variation of the method, fortunately, is low. With a confidence interval of around 10% (mean number of subjects n = 12) it is similar to the ISO sun protection factor test method (14).

SAND RESISTANCE

Many sunscreen formulations leave a sticky tacky film on the skin. As sunscreens are frequently used on sandy beaches, sand often stick to the skin, causing consumer discomfort. When the consumer brushes off the sand and with it some sunscreen, there are consequences as to the efficacy of the remaining sunscreen. Like water resistance in sunscreens, sand resistance in sunscreens is the ability of the sunscreen to retain its effectiveness while undergoing sand exposure. However, there is no formal ISO or Cosmetic Europe guideline as to how this can be measured. As described above, the water resistance SPF testing determines the SPF of a sunscreen product after a defined period of water exposure. Similarly, sand-resistance SPF testing should be a method to determine the SPF of a sunscreen product after a defined period of sand exposure.

Our clinical studies are designed to determine if sand that is poured onto the sunscreen-treated skin and brushed off has a statistically significant effect on the SPF of a sunscreen (19).

EYE PROTECTION

The eyes are our most important sensory organ. Ocular irritation testing represents an important step in the safety evaluation of cosmetic products. Increasing concern regarding the ethics of animal testing have prompted the development and use of numerous in vitro systems to approximate the ocular irritation potential of cosmetics such as sunscreens designed for human application. While these systems are capable of demonstrating potential tissue damage at a cellular level, human subjective responses to ocular cosmetic exposure like burning, which may be more or less sensitive to particular irritants, cannot be accurately predicted from in vitro results. Human ocular instillation represents a reliable, predictable and reproducible ocular irritant testing methodology to assess the safety of many substances and for determining the ocular irritant potential of sunscreen products in human eyes. Products such as sunscreens that are being used in or in the area around the eyes must be tested with regard to assessing their tolerability. The most commonly employed procedures are the eye-instillation method and the close-to-eye in-use test, and are performed by our in-house ophthalmologists.

QUALITY ASSURANCE AND GOOD TESTING PRACTICE

Requirements Regular participation in inter-laboratory tests is the best way to assure a permanently high level of quality testing over time. We participate every year in such round robin tests with good results and are querying why this opportunity is not taken up by other institutes. Inter-laboratory sunscreen tests are organized on a rotational basis by Bipea (International Bureau for Analytical Studies). Inter-laboratory comparisons were first organized by the DGK and COLIPA. Since 2009, BIPEA, founded in 1970 and specialized in the implementation and evaluation of international inter-laboratory comparisons, has taken over the organization of these tests. Together with establishing the proper functioning of our sun simulators through calibration by an external accredited service provider, we see the inter-laboratory tests as one of the most important key measures to assure outstanding quality in sunscreen testing.
Whilst in sunscreen testing ISO-guidelines for the core methods and a technical guidance paper for calibration of sun simulators by Cosmetics Europe (formerly Colipa) (20) are in place, and already help to establish good practice, this is not sufficient to establish good clinical testing practice. We have implemented - and recommend this to all who perform clinical sunscreen testing i.e. compliance with all appropriate main principles of ICH-GCP guidelines (21). Though these are only mandatory for clinical drug testing, important aspects can be adopted for cosmetic testing. Only the most important can be mentioned here:
An individual study protocol should be prepared to cover all details of the testing that are not covered by the guidelines;
An informed consent form should be signed by each volunteer after detailed oral and written information;
Electronic or paper Case Record Forms (CRF) should be used to document all procedures and results for each included volunteer;
Documentation should contain inclusion and exclusion criteria for volunteers and all adverse reactions observed by them and study personal.;
All raw data obtained should be recorded together with assessment time and signature of the assessor;
A detailed report with appropriate data appendices should be prepared that enables a detailed reconstruction of all study details.

CONCLUDING REMARKS

Amongst all the cosmetics currently available to consumers, sunscreens are probably the most effective class of protective products in terms of skin well-being, and their contribution to human health and safety is of great importance. High standards in sunscreen testing to ensure precise and reliable results are crucial to meet stringent safety requirements. Furthermore, since all the relevant testing-methods are by themselves complex in terms of in vivo designs, or are based on such methods, advanced standardization, high level of staff training, and a high frequency of result controls via round-robin-tests are essential to assure required high quality levels. Only high quality testing avoids imprecise testing results, which can lead to a misunderstanding of the protective function of sunscreen products and result in considerable damage to both consumers and producers.

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